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EXAMINER	
MYERS, CARLA J	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/625,124	DOBROWOLSKI ET AL.	
	Examiner	Art Unit	
	Carla Myers	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *03 March 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-24* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-24* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *23 July 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *8/9/2004*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Applicant's election with traverse of the G98:d7i3 mutation and the primers of SEQ ID NO: 4 and 9 and the probe of SEQ ID NO: 14 in the reply filed on March 3, 2006 is acknowledged. The traversal is on the ground(s) that the claims do not recite an improper Markush group because each of the mutations is present in the biotinidase and thereby share a common structure. It is also stated that the mutations all share a common utility in that they are biomarkers for identifying biotinidase deficiency.

These arguments have been fully considered but are not found persuasive because while each of the mutations shares the structure of containing a portion of the biotinidase gene, the mutations differ with respect to their nucleotide position and nucleotide identity. The G98:7di3 mutation involves the deletion of nucleotides GCGGCTTG, and the insertion of nucleotides TCC immediately 3-prime to a 12-bp polypyrimidine sequence in the BTD gene. The deletion/insertion results in a frameshift mutation that leads to a stop codon at amino acid 68, resulting in a truncated protein with an amino acid sequence different from that of biotinidase. On the other hand, the R538C mutation, for example, involves a C to T nucleotide substitution at nucleotide position 1612. This mutation may result in abnormal disulfide bond formation, causing rapid degradation of the aberrant enzyme. Thereby, each of the mutations are in fact structurally and functionally distinct from one another. Further, a sequence and literature search for G98:d7i3 is not co-extensive with a sequence and literature search for Q456H, R538C, D44H and A171T. Moreover, a finding that the G98:d7i3 mutation

is novel or unobvious over the prior art would not necessarily extend to a finding that the Q456H, R538C, D44H and A171T mutations are also novel and unobvious over the prior art. Similarly, a finding that the G98:d7i3 mutation is anticipated by or obvious in view of the prior art would not necessarily extend to a finding that the Q456H, R538C, D44H and A171T mutations are also anticipated by or obvious over the prior art. Further, the primers of SEQ ID NO: 4 and 9 and the probe of SEQ ID NO: 14 do not share sequence identity with the primers and probes of SEQ ID NO: 5-8, 10-13 and 20-23 and thereby do not in fact share a substantial structural feature.

The requirement is still deemed proper and is therefore made FINAL. Further, it is noted that the response did not specify the detector probe that is to be examined. However, since SEQ ID NO: 19 constitutes the detector probe for the G98:d7i3 mutation, this probe has also been examined here.

Accordingly, the claims have been examined only to the extent that they read on the G98:d7i3 mutation , the primers of SEQ ID NO: 4 and 9 and the probes of SEQ ID NO: 14 and 19. The remaining mutations, primers and probes have been withdrawn from consideration as being drawn to a non-elected invention.

Claim Rejections - 35 USC § 112

2. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-24 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-24 are indefinite because the claims do not recite a clear nexus between the preamble of the claims and the final process step of the claims. The claims are drawn to methods for detecting biotinidase deficiency. However, the final step is one for generating a melting curve. The claims do not clearly set forth how generating a melting curve results in the detection of biotinidase deficiency. Accordingly, it is unclear as to whether the claims are intended to be limited to methods for detecting biotinidase deficiency or methods for generating melting curves.

Claims 1-14 are indefinite over the recitation of "probe is adapted to match to a sequence" because it is unclear as to what is intended to be meant by this phrase. The specification does not provide a clear definition for what is intended to be meant by probes which match a sequence and there is no art recognized definition for this phrase. It is unclear, for instance, if probes which match a sequence are identical to a sequence, fully complementary to a sequence, share some unstated degree of identity with a sequence or share some unstated degree of complementarity to a sequence. Accordingly, one of skill in the art cannot determined the meets and bounds of the claimed subject matter.

Claims 1-14 are also indefinite because the claims refer to a "pair of labeled probes" and to a detection probe and to an anchor probe. However, the claims do not set forth the relationship between the pair of labeled probes and the detection and anchor probes. It is unclear as to whether the pair of probes consists of the detection and anchor probe or whether the pair of probes is distinct from the detection and anchor probes.

Claim 3 is indefinite over the recitation of "such amplification" because this language is vague. For instance, it is not clear as to whether "such amplification" refers to the previously stated amplification or refers to a different amplification that is similar in some unstated manner to the previous amplification. Similarly, claims 4-9 and 15-24 are indefinite over the recitation of "those such sequences."

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1 and 3-24 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for methods for detecting biotinidase deficiency in a newborn human wherein the method comprises performing the stated amplification and probe hybridization method and wherein detection of the presence of a G98:d7i3 biotinidase mutation indicates that the newborn is at risk of developing biotinidase deficiency, does not reasonably provide enablement for methods for detecting biotinidase deficiency in any organism by assaying for any mutation that is frequently observed in patients with biotinidase deficiency. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The following factors have been considered in formulating this rejection (*In re Wands,* 858F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988): the breadth of the claims, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art, the amount of direction or guidance

presented, the presence or absence of working examples of the invention and the quantity of experimentation necessary.

Breadth of the Claims:

The claims are drawn broadly to encompass methods for detecting biotinidase deficiency in a newborn by assaying for the presence of an amplification product "specific for detecting a mutation frequently observed in patients with biotinidase deficiency." The claims do not define the mutation in terms of the gene in which it is present, in terms of its nucleotide identity or in terms of its location within a gene. Thereby, the claimed mutations are not clearly defined in terms of either their structure or their function. While claims 4-13 and 15-24 define the primers and/or probes to be used to detect the mutation, these claims are also not limited to the detection of any particular mutation associated with biotinidase deficiency. Further, while claim 2 is drawn to methods which detect the mutations G98:d7i3, Q456H, R538C, D444H and A171T, the claim does not specify the gene in which these mutations are detected.

Nature of the Invention

The claims encompass methods for detecting biotinidase deficiency by assaying for a mutation. The invention is in a class of inventions which the CAFC has characterized as 'the unpredictable arts such as chemistry and biology" (Mycolgen Plant Sci., Inc. v. Monsanto Co., 243 F.3d 1316, 1330 (Federal Circuit 2001)).

Teachings in the Specification and State of the Art:

The specification (page 2) teaches that the mutations G98:d7i3, Q456H, R538C, D444H and A171T in the biotinidase gene are frequently found in patients which have

biotinidase activity. The teachings in the prior art have established that each of these mutations is associated with the occurrence of biotinidase deficiency and the detection of these mutations can be used to screen for individuals that are at an increased risk of having or developing biotinidase deficiency. However, the specification does not teach any additional mutations in the human biotinidase gene which are associated with biotinidase deficiency. Further, the specification and prior art do not teach any mutations in homologues of the biotinidase gene which could be used to detect biotinidase deficiency in non-human organisms. There are also no apparent teachings in the specification or prior art of mutations in other genes which could be detected as indicative of biotinidase deficiency. Accordingly, while the specification has enabled methods which detect the presence of the G98:d7i3, Q456H, R538C, D444H and A171T biotinidase mutations, the specification has not enabled methods which detect any mutation in any gene wherein the mutation is frequently observed in patients with biotinidase deficiency.

The Predictability or Unpredictability of the Art and Degree of Experimentation:

The art of identifying novel variants in the biotinidase gene or other genes which are sufficiently biotinidase deficiency is highly unpredictable. Knowledge of the sequence of the wildtype biotinidase gene and other genes does not allow one to immediately envision specific mutations that frequently occur in patients having biotinidase deficiency. Once a new mutation is identified, it remains unpredictable as to whether that mutation is sufficiently linked with biotinidase deficiency to be informative and to be diagnostic for biotinidase deficiency. Further, once a gene has been identified,

it remains unpredictable as to whether other mutations in the gene will be associated with a disease or phenotype.

Without extensive information regarding the structure-function relationship between the biotinidase gene and other genes and the occurrence of biotinidase deficiency, it is highly unpredictable as to what would be the identity of additional mutant, allelic, or splice variants which would be associated with biotinidase deficiency. Thus, one cannot readily anticipate the effect of a polymorphism or mutation within the biotinidase gene or other genes.

Amount of Direction or Guidance Provided by the Specification:

The specification teaches only 5 mutations in the biotinidase gene which are associated with biotinidase deficiency. To identify additional variants of the biotinidase gene which are diagnostic would require extensive experimentation. For example, such experimentation may involve sequencing the biotinidase gene of affected individuals having biotinidase deficiency, sequencing the biotinidase gene of control individuals, comparing the sequences of these two groups, and then identifying variations which are present only in the affected group and not in the control group. Such random, trial by error experimentation is considered to be undue. Further, extensive experimentation would be required to identify additional genes which may be linked in some manner to biotinidase deficiency and to identify novel mutations in these genes which could be used to diagnose biotinidase deficiency in newborns.

While methods for identifying mutations are known in the art, such methods provide only the general guidelines that allow researchers to randomly search for

mutations that may linked to a disease. The results of performing such methodology is highly unpredictable. The specification has provided only an invitation to experiment. The specification does not provide a predictable means for identifying additional variants of the biotinidase gene and using these variants to screen for biotinidase deficiency.

Additionally, the specification and prior art do not teach the presence of mutations in homologues of the biotinidase gene and do not teach the detection of biotinidase deficiency in non-human organisms. It is highly unpredictable as to whether the mutations which occur in the human biotinidase gene will also occur in non-human organisms and whether these mutations could be used to detect a form of biotinidase deficiency in other organisms. Accordingly, the specification does not provide sufficient guidance as to how to apply the claimed method to the detection of biotinidase deficiency in non-human organisms.

Working Examples:

Again, the specification exemplifies methods for detecting the presence of the G98:d7i3, Q456H, R538C, D444H and A171T biotinidase mutations as indicative of biotinidase deficiency. There are no additional examples provided in the specification in which mutations in other genes or in which other mutations in the biotinidase gene are defected as indicative of biotinidase activity. There are also no examples provided of mutations in non-human organisms which are associated with biotinidase activity and no examples in which biotinidase deficiency is detected in non-human organisms.

Conclusions:

Case law has established that '(t)o be enabling, the specification of a patent must teach those skilled in the art how to make and use the full scope of the claimed invention without 'undue experimentation.'" *In re Wright* 990 F.2d 1557, 1561. *In re Fisher,* 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970) it was determined that '(t)he scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification to persons of ordinary skill in the art". The amount of guidance needed to enable the invention is related to the amount of knowledge in the art as well as the predictability in the art. Furthermore, the Court in *Genetech Inc. v Novo Nordisk* 42 USPQ2d 1001 held that '(I)t is the specification, not the knowledge of one skilled in the art that must supply the novel aspects of the invention in order to constitute adequate enablement".

In the instant case, the claims do not bear a reasonable correlation to the scope of enablement because the specification teaches only 5 mutations in the biotinidase gene which are associated with biotinidase deficiency. The specification does not teach a representative number of additional variants, including insertions, deletions, substitutions or splice variants in the biotinidase gene or in other genes which are associated with biotinidase deficiency. Also, the specification does not teach a representative number of organisms other than humans which could be diagnosed for biotinidase deficiency. Accordingly, although the level of skill in the art of molecular biology is high, given the lack of disclosure in the specification and in the prior art and the unpredictability of the art, it would require undue experimentation for one of skill in the art to make and use the invention as broadly claimed.

4. Claims 1-24 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a Written Description rejection.

The claims are drawn broadly to encompass methods for detecting biotinidase deficiency in a newborn by assaying for the presence of an amplification product "specific for detecting a mutation frequently observed in patients with biotinidase deficiency." The claims do not define the mutation in terms of the gene in which it is present, in terms of its nucleotide identity or in terms of its location within a gene. Thereby, the claimed mutations are not clearly defined in terms of either their structure or their function. While claims 4-13 and 15-24 define the primers and/or probes to be used to detect the mutation, these claims are also not limited to the detection of any particular mutation associated with biotinidase deficiency. Further, while claim 2 is drawn to methods which detect the mutations G98:d7i3, Q456H, R538C, D444H and A171T, the claim does not specify the gene in which these mutations are detected.

The specification teaches that the mutations G98:d7i3, Q456H, R538C, D444H and A171T in the biotinidase gene are frequently found in patients which have biotinidase activity. The teachings in the prior art have established that each of these mutations is associated with the occurrence of biotinidase deficiency and the detection of these mutations can be used to screen for individuals that are at an increased risk of having or developing biotinidase deficiency. Accordingly, while methods which detect

the presence of the G98:d7i3, Q456H, R538C, D444H and A171T biotinidase mutations meet the written description requirements of 35 U.S.C. 112, first paragraph, the specification does not disclose and fully characterize the genus required by the claims of any mutation frequently observed in patients with biotinidase deficiency.

Vas-Cath Inc. V. Mahurkar, 19 USPQ2d 1111, clearly states that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed". Applicant is reminded that *Vas-Cath* makes clear that the written description provision of 35 U.S.C. 112 is severable from its enablement provision. In *The Regents of the University of California v. Eli Lilly* (43 USPQ2d 1398-1412), the court held that a generic statement which defines a genus of nucleic acids by only their functional activity does not provide an adequate written description of the genus. The court indicated that while Applicants are not required to disclose every species encompassed by a genus, the description of a genus is achieved by the recitation of a representative number of DNA molecules, usually defined by a nucleotide sequence, falling within the scope of the claimed genus. At section B(1), the court states that "An adequate written description of a DNA...'requires a precise definition, such as by structure, formula, chemical name, or physical properties', not a mere wish or plan for obtaining the claimed chemical invention".

In analyzing whether the written description requirement is met for a genus claim, it is first determined whether a representative number of species have been described by their complete structure. In the instant case, 5 members of the genus of mutations

have been identified. No additional nucleotide variations have been disclosed. It is then determined whether a representative number of species have been sufficiently described by other relevant identifying characteristics (e.g. restriction map, biological activity of an encoded protein product, etc.). In the instant case, no such identifying characteristics have been provided for any additional mutations that are frequently observed in patients having biotinidase deficiency. However, the claims as written are inclusive of a potentially large genus of mutations in the biotinidase gene or any other gene that may be currently known or unknown and in which mutations may be identified which are present in patients with biotinidase deficiency. While one could contemplate a nucleotide substitution, deletion or addition at each and every position in the biotinidase gene and in other genes that may be linked in some manner to biotinidase deficiency, such nucleotide variations are not considered to be equivalent to specific nucleotide variations associated with biotinidase deficiency. Rather, mutations that are associated with biotinidase deficiency represent a distinct group of nucleotide variations which are expected to occur at only specific locations within a given gene and consist of specific nucleotide alterations. Accordingly, knowledge of the sequence of the wild-type biotinidase gene or of other genes in the human genome or in other non-human genomes does not allow the skilled artisan to envision all of the contemplated mutations encompassed by the claimed genus. Conception of the claimed invention cannot be achieved until reduction to practice has occurred, regardless of the complexity or simplicity of potential methods for isolating additional nucleotide variations. As stated in *Fiers v. Revel*, 25 USPQ2d 1601, 1606 (CAFC 1993) and *Amgen Inc. V. Chugai*

Pharmaceutical Co. LTD, 25 USPQ2d 1016, one cannot describe what one has not conceived.

Accordingly, the disclosure in the specification of 5 mutations in the biotinidase gene is not considered to constitute a representative number of nucleotide variants, including insertions, deletions, substitutions or splice variants, in any exon, intron or non-coding region of the biotinidase gene or in any other uncharacterized gene that might be associated with the occurrence of biotinidase deficiency. For these reasons, Applicants have not provided sufficient evidence that they were in possession, at the time of filing, of the invention as it is broadly claimed and thus the written description requirement has not been satisfied for the claims as they are broadly written. Applicants attention is drawn to the Guidelines for the Examination of Patent Applications under 35 U.S.C. 112, ¶ 1 "Written Description" Requirement, Federal Register, Vol. 66, No. 4, pages 1099-1111, Friday January 5, 2001.

Claim Rejections - 35 USC § 103

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein

were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1 and 2 are rejected under 35 U.S.C. 103(a) as being unpatentable over Pals (Journal Bioch Biophys Methods 2001. 47: 121-129) in view of Pomponio (Human Molecular Genetics. 1997. 6: 739-745; cited in the IDS).

Pals (page 123-124) teaches a method for detecting a mutation in a sample nucleic acid comprising: i) amplifying a DNA strand from a specimen to form an amplification product, wherein the DNA strand contains a mutation; ii) hybridizing a pair of probes to the amplification product, wherein one of the probes is a detection probe that hybridizes to the mutation and the other probe is an anchor probe that hybridizes adjacent to the detection probe to form hybrids; iii) allowing fluorescence resonance energy transfer to occur between a donor fluorophore on one of the probes and an acceptor fluorophore on the other probe; iv) generating a melting curve with peaks for the melting temperature of each hybrid; v) comparing the melting curves to those of reference nucleic acids which either comprise the mutation or do not comprise the mutation; and vi) based on the comparison, detecting the presence of the mutation in the sample nucleic acid.

Pals (see abstract) states that "(f)or known mutations, real time polymerase chain reaction followed by melting curve analysis, using hybridization probes, is highly

sensitive, rapid and an efficient approach to mutation detection." The reference (page 129) also teaches that the methodology can be used to detect mutations in small quantities of DNA and in single cells. Pals exemplifies methods in which mutations in the BRCA1 gene are detected, but teaches that this methodology is not limited to just the BRCA1 gene. As stated by Pals, "the technique has great potential in the fields where reliable and extremely fast mutation detection is important, such as pre-implantation diagnosis" (see page 128). Pals does not specifically exemplify methods in which a mutation is detected in the biotinidase gene.

However, Pomponio teaches methods for detecting mutations in the biotinidase gene (page 744). In particular, Pomponio teaches methods for detecting the G98:d7i3 mutation and teaches that this mutation is associated with biotinidase deficiency (page 741). The reference (page 739) states that "it is important that biotinidase deficiency is diagnosed early sot that biotin therapy can be initiated before permanent neurological damage occurs."

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have applied the method of Pals to the detection of the G98:d7i3 biotinidase mutation in order to have provided a reliable, rapid and sensitive means for detecting biotinidase deficiency in newborns, and to thereby allow for early initiation of therapy in newborns carrying the G98:d7i3 mutation.

6. Claims 4-15 and 17-24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Pals in view of Pomponio and further in view of Knight (GenBank Accession No. AF018631) and Knight (Mammalian Genome. 1998. 9: 327-330).

The teachings of Pals and Pomponio are presented above. While the combined reference teach the detection of the G98:di73 biotinidase mutation using a set of primers to amplify the sequences comprising this region and a set of detector and anchor probes, the combined references do not teach performing the detection method using primers consisting of SEQ ID NO: 4 and 9, a detector probe consisting of SEQ ID NO: 19 and an anchor probe consisting of SEQ ID NO: 14.

However, Pomponio does teach amplifying the biotinidase nucleic acids using a primer ("337.A", page 744) which differs from that of present SEQ ID NO: 9 in that it is missing a T at the 3' end:

337.A: 5'-CTCATACACGGCAGCCACA-3'

SEQ ID NO : 9 : 5'-CTCATACACGGCAGCCACAT-3'

Further, the complete sequence of the biotinidase gene was known at that the time the invention was made and is specifically set forth in GenBank Accession No. AF018631. Also, Knight (page 328; Figure 1) teaches the intron/exon boundaries of the biotinidase gene, including the 3' and 5' splice sites. In particular, Knight (Figure 1) teaches the sequence of the 3' splice site of intron 1, which differs from the present primer of SEQ ID NO: 4 in that it is missing a G at the 5' end and has an extra T at the 3' end:

3' splice site: 5'-CCCATTACATTCCAGATTTGT-3'

SEQ ID NO: 4: 5'-GCCCATTACATTCCAGATTTG-3'

Moreover, Pals provides extensive guidance as to how to select primers for amplifying the target sequence containing a mutation and as to how to select probes

that hybridize to the amplified region, which detect the presence of the mutation and which interact by FRET to produce a detectable signal (see pages 122-123). Pals teaches the selection of detection and anchor probes so that the detection probe hybridizes to the mutation and so that the probes have a melting temperature at least 5°C above the melting temperature of the primers and the anchor probe has a melting temperature at least 5°C above that of the detection probe (see page 123). Pals (page 122) also teaches the selection of probes that are 1 to 5 bases apart, so that the 3' end label of the 5' probe is in close proximity to the 5' end label of the 3' probe when the pair of probes is hybridized to the target nucleic acid. Thereby, the parameters and objectives involved in the selection of primers and probes were well known in the art at the time the invention was made. Moreover, software programs were readily available which aid in the identification of conserved and variable sequences and in the selection of optimum primer pairs and probe pairs. The prior art is replete with guidance and information necessary to permit the ordinary artisan to design additional primers and probes for the amplification of the biotinidase G98:d7i3 mutation. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have generated primers and probes, including the primers of SEQ ID NO: 4 and 9 and the probes of SEQ ID NO: 14 and 19, in order to have provided primers and probes that could be used in the method of Pals for the rapid and efficient detection of the biotinidase G98:d7i3 mutation.

Regarding claims 7 and 19, Pals (page 123) teaches that the detection probe is labeled with LC red640.

Regarding claims 8 and 20, Pals (see page 123 and the exemplified "probe 2") teaches that the detection probe is phosphorylated.

Regarding claims 9 and 21, Pals teaches that the anchor probe is labeled with fluorescein (i.e., FITC).

Regarding claims 11-13 and 22-24, Pals does not teach labeling the detection probe with FITC and the anchor probe with LC red640 and phosphorylating the 3' end of the anchor probe. However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have alternatively labeled the detection probe with FITC and the anchor probe with LC red640 and to have phosphorylated the anchor probe in order to have performed assays in which the anchor and detection probes hybridized in the opposite order (i.e., with the detection probe 5' to the anchor probe) to the target nucleic acid.

Regarding claim 14, Pals teaches that the melting curve temperature is plotted over a temperature range of 45 to 80°C, but does not teach that the melting curve is plotting over a range of 35 to 76°C. However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified and optimized the range of the temperature for the melting curve according to the melting temperatures of the probes, and thereby to have used a temperature range of 35 to 76°C for the probes of SEQ ID NO: 14 and 19, in order to have provided the most accurate and sensitive method for detecting the G98:d7i3 mutation.

7. Claims 3 and 16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Pals in view of Pomponio and further in view of Wittwer (U.S. Patent No. 6,174,670).

The teachings of Pals and Pomponio are presented above. The combined references teach PCR amplification of the target nucleic acid using primers that amplify a sequence comprising a mutation to be detected. The combined references do not teach asymmetric PCR amplification of the target nucleic acid.

However, Wittwer teaches methods of real-time PCR amplification and detection of target nucleic acids using an anchor and detection probe labeled with FRET moieties (see Figure 18 and col. 4-5). Wittwer (see, for example, col. 44 and 46) teaches that PCR may be performed in an asymmetric manner using approximately twice the quantity of the forward primer, versus the reverse primer.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to have further modified the method of Pals and Pomponio so as to have performed the PCR step in an asymmetric manner in order to have generated excess quantities of the strand of target nucleic acid to which the anchor and detection probes were to hybridize to thereby facilitate the detection of the G98:d7i3 mutation.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Carla Myers whose telephone number is (571) 272-0747. The examiner can normally be reached on Monday-Thursday from 6:30 AM-5:00 PM. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla, can be reached on (571)-272-0735.

The fax phone number for the organization where this application or proceeding is assigned is (571)-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at (866)-217-9197 (toll-free).

Carla Myers
March 28, 2006

CARLA J. MYERS
PRIMARY EXAMINER